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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of :

Masataka NADAOKA et al. : **Attn: BOX PCT**

Serial No. NEW : **Docket No. 2002-0074A**

Filed January 25, 2002 :

BIOSENSOR
[Corresponding to PCT/JP01/04419
Filed May 25, 2001]

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents,
Washington, DC 20231

Sir:

Prior to examination of the above-referenced U.S. patent application please amend the application as follows:

IN THE SPECIFICATION

Please amend the specification as follows:

Please replace the paragraph beginning at page 10, line 12, to line 22, with the following rewritten paragraph:

According to the present invention, the biosensor further includes a specimen holding part for holding the inspection target solution so as to be in contact with the cavity part. Therefore, an operation for moving the inspection target solution to another container after collecting the inspection target solution or an operation for quantitatively measuring a predetermined volume of collected inspection target solution is not required, and the inspection target solution can be held in the specimen holding part, thereby reducing outside pollution by the specimen, resulting in a safe, sanitary, quick, and simple measurement with high accuracy.

Please replace the paragraph beginning at page 17, line 5, to page 18, line 7, with the following rewritten paragraph:

Further, the space forming material 8 may be provided with an air vent for assisting the flowing-in of the inspection target solution in the cavity part 1. According to the shape of the air forming material 8, ends of the cavity part 1 other than the end to which the inspection target solution is attached may be blocked with side walls, and in this case, even when a specimen is attached to the opened end of the cavity part 1, air existing in the cavity part 1 does not or hardly comes out of the cavity part 1 before the specimen is introduced, whereby the introduction of the inspection target solution to the cavity part 11 is delayed or the introduction is not performed at all, resulting in a low-accuracy measurement result or an erroneous measurement result. On the other hand, when the air vent is provided in the space forming material 8, air in the cavity part 1 can come out and the specimen easily flows in the cavity part 1, thereby preventing occurrence of an adverse effect on the measurement. While this air vent is preferred to be provided at a backmost part of the cavity part, seen from the direction of the inspection target solution flowing-in, it may be provided at parts other than the backmost part according to need. As a material of the space forming material 8, a synthetic resin material such as ABS, polystyrene, and polyvinyl chloride, as well as a solution-impermeable material such as metal and glass can be employed, and, while the material is preferably transparent or translucent to check on the flowing-in of the inspection target solution into the cavity part 1, if not transparent, a colored or opaque material is also available.

Please replace the paragraph beginning at page 19, line 18, to page 20, line 9, with the following rewritten paragraph:

Further, sodium percarbonate can be also held in the cavity part 1 as a reagent component which has an bleaching action according to need. By providing such bleaching reagent part, a so-called background, which is generated due to a color of the inspection target solution and occurs with respect to the reagent immobilization part 5 for reading a reaction, is reduced, resulting in an increase in an S/N ratio, whereby a simple and high-accuracy measurement result can be obtained.

The background here is a color having no relation to the reaction, and there is a phenomenon where such color remains in the whole development layer including the reagent immobilization part in a case, for example, where the inspection target solution itself is colored. Further, the S/N ratio in this case is a ratio of a signal obtained by the marker reagent in the reaction to a so-called noise which does not derive from the marker reagent but arises from the background in a part other than the development layer.

Please replace the paragraph beginning at page 40, line 6, to page 41, line 6, with the following rewritten paragraph:

Further, according to the present invention, when the same biosensor as the biosensors described in the first to third embodiments is a biosensor employed for a one-step immuno-chromatography, in the one-step immuno-chromatography which is prevailing in the market as a simplified immunoassay method, a user does not have to quantitatively measure the volume of the inspection target solution previously, and the amount of the inspection target solution can be confirmed at measurement, thereby reducing the erroneous judgement and realizing a high-accuracy and simplified operation held by a conventional one-step immuno-chromatography. The one-step immuno-chromatography is an immunoassay method in which a measurement is started by the application of the inspection target solution employing a permeable porous material, and is a system of measurement which utilizes the antigen antibody reaction and in which the B/F separation is implemented in the process of the inspection target solution permeating a chromatography carrier, while a washing operation such as the B/F separation is required in a usual immunoassay method. The whole reagent is usually in a dry state and is permeated by the inspection target solution at measurement. This is referred to as one-step immuno-chromatography since a basic measurement operation by a user is only to apply the inspection target solution. While a gold colloid and a latex are typical as markers, magnetic particles, an enzyme, a metallic colloid, or the like are also used.

IN THE CLAIMS

Please amend the claims as follows:

3. (Amended) The biosensor as defined in Claim 1, wherein

the cavity part temporarily holds the inspection target solution.

4. (Amended) The biosensor as defined in Claim 1, wherein

the cavity part defines the amount of the flowing-in of the inspection target solution by the volume of the cavity part.

5. (Amended) The biosensor as defined in Claim 1, wherein

the cavity part has a volume for the flowing-in of the inspection target solution enough to develop in the development layer.

6. (Amended) The biosensor as defined in Claim 1 further including

a cell component destruction reagent part for destroying cell components in the cavity part.

7. (Amended) The biosensor as defined in Claim 1 further including

a cell component shrinkage reagent part for shrinking cell components in the cavity part.

8. (Amended) The biosensor as defined in Claim 1 further including

a bleaching reagent part in the cavity part.

9. (Amended) The biosensor as defined in Claim 1, wherein

the cavity part has a volume of 20μl (microliter) or less.

10. (Amended) The biosensor as defined in Claim 1, wherein

the cavity part has a means for externally checking on flowing-in of the inspection target solution.

11. (Amended) The biosensor as defined in Claim 1, wherein

the space forming part is partially or entirely light permeable.

12. (Amended) The biosensor as defined in Claim 1 further including

a separation part for separating concrete components unnecessary for a measurement in the cavity part.

13. (Amended) The biosensor as defined in Claim 1 further including

a specimen holding part for holding the inspection target solution so as to be in contact with the cavity part.

14. (Amended) The biosensor as defined in Claim 1, wherein

the specimen holding part holds a larger amount of inspection target solution than the volume of the cavity part.

15. (Amended) The biosensor as defined in Claim 1, wherein

the bottom surface of the specimen holding part is as high as or higher than that of the cavity part.

17. (Amended) The biosensor as defined in Claim 1 further including

an air vent for assisting the flowing-in of the inspection target solution in the cavity part.

18. (Amended) The biosensor as defined in Claim 1 further including

a porous material which can be permeated by permeation of the inspection target solution in the cavity part.

19. (Amended) The biosensor as defined in Claim 1, wherein

whole reagents including the reagent in the reagent immobilization part and the marker reagent are in a dry state and they are entirely in a dry state.

20. (Amended) The biosensor as defined in Claim 1, wherein

the biosensor is employed for an immuno-chromatography.

21. (Amended) The biosensor as defined in Claim 1, wherein

the biosensor is employed for a one-step immuno-chromatography

Please add the following new claims:

22. The biosensor as defined in Claim 2, wherein

the cavity part temporarily holds the inspection target solution.

23. The biosensor as defined in Claim 2, wherein

the cavity part defines the amount of the flowing-in of the inspection target solution by the volume of the cavity part.

24. The biosensor as defined in Claim 2, wherein

the cavity part has a volume for the flowing-in of the inspection target solution enough to develop in the development layer.

25. The biosensor as defined in Claim 2 further including

a cell component destruction reagent part for destroying cell components in the cavity part.

26. The biosensor as defined in Claim 2 further including

a cell component shrinkage reagent part for shrinking cell components in the cavity part.

27. The biosensor as defined in Claim 2 further including a bleaching reagent part in the cavity part.

28. The biosensor as defined in Claim 2, wherein the cavity part has a volume of 20μl (microliter) or less.

29. The biosensor as defined in Claim 2, wherein the cavity part has a means for externally checking on flowing-in of the inspection target solution.

30. The biosensor as defined in Claim 2, wherein the space forming part is partially or entirely light permeable.

31. The biosensor as defined in Claim 2 further including a separation part for separating concrete components unnecessary for a measurement in the cavity part.

32. The biosensor as defined in Claim 2 further including a specimen holding part for holding the inspection target solution so as to be in contact with the cavity part.

33. The biosensor as defined in Claim 2, wherein the specimen holding part holds a larger amount of inspection target solution than the volume of the cavity part.

34. The biosensor as defined in Claim 2, wherein the bottom surface of the specimen holding part is as high as or higher than that of the cavity part.

35. The biosensor as defined in Claim 32, wherein

the cavity part has a volume of 100μl or less.

36. The biosensor as defined in Claim 2 further including

an air vent for assisting the flowing-in of the inspection target solution in the cavity part.

37. The biosensor as defined in Claim 2 further including

a porous material which can be permeated by permeation of the inspection target solution in the cavity part.

38. The biosensor as defined in Claim 2, wherein

whole reagents including the reagent in the reagent immobilization part and the marker reagent are in a dry state and they are entirely in a dry state.

39. The biosensor as defined in Claim 2, wherein

the biosensor is employed for an immuno-chromatography.

40. The biosensor as defined in Claim 2, wherein

the biosensor is employed for a one-step immuno-chromatography.

REMARKS

The present Preliminary Amendment is submitted to make minor editorial changes so as to generally improve the form of the specification and to delete the multiple dependency of the claims, thereby placing such claims in condition for examination and reducing the required PTO filing fee.

Attached hereto is a marked-up version of the changes made to the [specification, claims and abstract] by the current [Preliminary Amendment]. The attached page is captioned "**Version with Markings to Show Changes Made**".

Respectfully submitted,

Masataka NADAOKA et al.

By

Nils E. Pedersen
Registration No. 33,145
Attorney for Applicants

NEP/krl
Washington, D.C. 20006-1021
Telephone (202) 721-8200
Facsimile (202) 721-8250
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of inspection target solution, resulting in a higher-accuracy measurement.

According to the present invention, the biosensor further includes a separation part for separating concrete components unnecessary for a measurement in the cavity part. Therefore, concrete components in the inspection target solution which are unnecessary for a measurement can be separated, and there is no need to previously eliminate the concrete components in the inspection target solution which are unnecessary for the measurement by an operation of centrifugation, filtration, or the like, resulting in a simple and high-accuracy measurement.

According to the present invention, the biosensor further includes a specimen holding part for holding the inspection target solution so as to be in contact with the cavity part. Therefore, an operation for moving the inspection target solution to another container [for] after collecting the inspection target solution or an operation for quantitatively measuring a predetermined volume of collected inspection target solution is not required, and the inspection target solution can be held in the specimen holding part, thereby reducing outside pollution by the specimen, resulting in a safe, sanitary, quick, and simple measurement with high accuracy.

According to the present invention, in the biosensor, the specimen holding part holds a larger amount of inspection target solution than the volume of the cavity part. Therefore,

protecting from pollution by the improvident inspection target solution being attached or scattering to the outside, when a biosensor to which the inspection target solution has been applied is handled.

Further, the space forming material 8 may be provided with an air vent for assisting the flowing-in of the inspection target solution in the cavity part 1. According to the shape of the air forming material 8, ends of the cavity part 1 other than the end to which the inspection target solution is attached may be blocked with side walls, and in this case, even when a specimen is attached to the opened end of the cavity part 1, air existing in the cavity part 1 does not or hardly comes out of the cavity part 1 before the specimen is introduced, whereby the introduction of the inspection target solution to the cavity part 11 is delayed or the introduction is not performed at all, resulting in a low-accuracy measurement result or an erroneous measurement result. On the other hand, when the air vent is provided in the space forming material 8, air in the cavity part 1 can come out and the specimen easily flows in the cavity part 1, thereby preventing occurrence of an adverse effect on the measurement. While this air vent is preferred to be provided at a backmost part of the cavity part, seen from the direction of the inspection target solution flowing-in, it may be provided at parts other than the backmost part according to need. As a material of the space

forming material 8, a synthetic resin material such as ABS, polystyrene, and polyvinyl chloride, as well as a solution-impermeable material such as metal and glass can be employed, and, while the material is preferably transparent or translucent to check on the flowing-in of the inspection target solution into the cavity part 1, if not transparent, a colored or opaque material is also available.

The cavity part 1 is formed by arranging the space forming material 8 on the development layer 2, and the cavity part 1 is adjacent to the development layer 2, so that the inspection target solution flows in the cavity part 1 and starts permeating the development layer 2, thereby performing a measurement. To perform a high-accuracy measurement when the inspection target solution is small in amount or a small and unknown amount of inspection target solution which is collected with a blood collecting puncture device is measured, the cavity part 1 preferably has the volume of 20μl (microliter) or less.

In a case where the inspection target solution having cell components is taken as a specimen, a cell shrinkage reagent is preferably held in the cavity part 1, and potassium chloride is employed as the cell concentration reagent, for example. By providing this cell shrinkage reagent, even when the inspection target solution having cell components is employed, a measurement operation can be performed easily without quantitatively measuring a predetermined volume of inspection

target solution employing a high-accuracy dispenser or the like, and a high-accuracy measurement result can be obtained without clogging the development layer with the cell components due to an action of a cell component shrinker. The cell shrinkage reagent is a reagent provided when the cell components are included in the inspection target solution, and is not particularly required when the inspection target solution including no cell components is employed. Further, the cell component shrinker may be an inorganic compound including salt, such as inorganic salt other than the potassium chloride, natrium chloride, or sodium phosphate, amino acid such as glycine or glutamic acid, imino acid such as proline, sugars such as glucose, sucrose, or trehalose, and sugar alcohol such as glucitol in the same way, as long as they have effects of shrinking cells. A system including such cell component shrinker is particularly effective when whole blood is employed as the inspection target solution.

Further, sodium percarbonate can be also held in the cavity part 1 as a reagent component which has an bleaching action according to need. By providing such bleaching reagent part, a so-called background, which is generated due to a color of the inspection target solution and occurs with respect to the reagent immobilization part 5 for reading a reaction, is reduced, resulting in an increase in an S/N ratio, whereby a simple and high-accuracy measurement result can be obtained.

The background here is a color having no relation to the reaction, and there is a phenomenon where such color remains in the whole development layer including the reagent immobilization part in a case, for example, where the inspection target solution itself is colored. Further, the S/N ratio in this case is a ratio of a signal obtained by the marker reagent in the reaction to a so-called noise which does not derive from the marker reagent but arises from the background in a part other than the ~~reactive~~ development layer.

Figures 2 are diagrams illustrating an example of the biosensor shown in figures 1, which is provided with a separation layer; figure 2(a) is an exploded view of the biosensor and figure 2(b) is a perspective view of the biosensor. The same constitutions as those shown in figures 1 are denoted by the same reference numerals, and their descriptions will be omitted.

Numeral 9 denotes a separation layer which can separate concrete components unnecessary for a measurement, and is composed of glass fiber filter paper. The glass fiber filter paper is employed as the separation layer 9 as an example, and any arbitrary materials which can be penetrated by the inspection target solution, such as a nonwoven fabric, filter paper, a glass fiber, a membrane filter, and a cloth, are available, as for the material employed for the development layer 2. Particularly, when a porous material which can be

measurement. While a gold colloid and a latex are typical as markers, magnetic particles, an enzyme, a metallic colloid, or the like are also used. When the marker is an enzyme, there is included an operation of a user adding an enzyme substrate or a reaction stop reagent as a measurement operation.

Further, according to the present invention, when the same biosensor as the biosensors described in the first to third embodiments is a biosensor employed for a one-step immuno-chromatography, in the one-step immuno-chromatography which is prevailing in the market as a simplified immunoassay method, a user does not have to quantitatively measure the volume of the inspection target solution previously, and the amount of the inspection target solution can be confirmed at measurement, thereby reducing the erroneous judgement and realizing a high-accuracy and simplified operation held by a conventional one-step immuno-chromatography. The one-step immuno-chromatography is an immunoassay method in which a measurement is started by the application of the inspection target solution employing a permeable porous material, and is a system of measurement which utilizes the antigen antibody reaction and in which the B/F separation is implemented in the process of the inspection target solution permeating a chromatography carrier, while a washing operation such as the B/F separation is required in a usual immunoassay method. The whole reagent is usually in a dry state and is permeated by the inspection target solution at

measurement. This is referred to as one-step immuno-chromatography since a basic measurement operation ~~of~~ by a ~~use difference~~ user is only to apply the inspection target solution. While a gold colloid and a latex are typical as markers, magnetic particles, an enzyme, a metallic colloid, or the like are also used.

Next, the present invention will be described more specifically through examples. The present invention is not restricted to descriptions in these examples in the scope of the present invention.

(Example 1)

Creation of test strip for measuring hCG in urine

The immuno-chromatography development layer 2 which includes an anti-hCG-ß antibody immobilization line and a broad band of a complex of an anti-hCG-α antibody and gold colloid in a nitrocellulose film was manufactured. The cavity part 1 was formed by the space forming material 8 in a part where the inspection target solution is applied on this immuno-chromatography development layer 2, thereby manufacturing an immuno-chromatography test strip. This test strip has the structure as shown in figures 1. According to figures 1, the test strip includes the antibody immobilization part 5 and the marker reagent holding part 4 located on the side of contacting place of the inspection target solution with respect to the antibody immobilization part, which includes the complex of the

which can be eluted by flowing-in of the inspection target solution, in the cavity part.

3. The biosensor as defined in Claim 1 or 2, wherein

the cavity part temporarily holds the inspection target solution.

4. The biosensor as defined in Claim 1 or 2, wherein

the cavity part defines the amount of the flowing-in of the inspection target solution by the volume of the cavity part.

5. The biosensor as defined in Claim 1 or 2, wherein

the cavity part has a volume for the flowing-in of the inspection target solution enough to develop in the development layer.

6. The biosensor as defined in Claim 1 or 2 further including

a cell component destruction reagent part for destroying cell components in the cavity part.

7. The biosensor as defined in Claim 1 or 2 further including

a cell component shrinkage reagent part for shrinking cell components in the cavity part.

8. The biosensor as defined in Claim 1 or 2 further including

a bleaching reagent part in the cavity part.

9. The biosensor as defined in Claim 1 or 2, wherein the cavity part has a volume of 20 μl (microliter) or less.

10. The biosensor as defined in Claim 1 or 2, wherein the cavity part has a means for externally checking on flowing-in of the inspection target solution.

11. The biosensor as defined in Claim 1 or 2, wherein the space forming part is partially or entirely light permeable.

12. The biosensor as defined in Claim 1 or 2 further including a separation part for separating concrete components unnecessary for a measurement in the cavity part.

13. The biosensor as defined in Claim 1 or 2 further including a specimen holding part for holding the inspection target solution so as to be in contact with the cavity part.

14. The biosensor as defined in Claim 1 or 2, wherein the specimen holding part holds a larger amount of inspection target solution than the volume of the cavity part.

15. The biosensor as defined in Claim 1 $\boxed{\text{or 2}}$, wherein the bottom surface of the specimen holding part is as high as or higher than that of the cavity part.

16. The biosensor as defined in Claim 13, wherein the cavity part has a volume of 100μl or less.

17. The biosensor as defined in Claim 1 $\boxed{\text{or 2}}$ further including an air vent for assisting the flowing-in of the inspection target solution in the cavity part.

18. The biosensor as defined in Claim 1 $\boxed{\text{or 2}}$ further including a porous material which can be permeated by permeation of the inspection target solution in the cavity part.

19. The biosensor as defined in Claim 1 $\boxed{\text{or 2}}$, wherein whole reagents including the reagent in the reagent immobilization part and the marker reagent are in a dry state and they are entirely in a dry state.

20. The biosensor as defined in Claim 1 $\boxed{\text{or 2}}$, wherein the biosensor is employed for an immuno-chromatography.

21. The biosensor as defined in Claim 1 $\boxed{\text{or 2}}$, wherein the biosensor is employed for a one-step immuno-

chromatography.